

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (previously presented) An RNA higher-order structure having a function for promoting a translation activity which comprises a base sequence selected from the group consisting of:

- 1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;
- 2) a base sequence containing the base sequence of 1);
- 3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;
- 4) a complementary strand of the base sequences of 1) to 3);
- 5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and
- 6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity.

2. (original) The RNA higher-order structure according to claim 1 wherein at least PK (pseudoknot) I, II, and III structures are maintained therein.

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

3. (previously presented) A recombinant vector containing a polynucleotide that comprises at least one base sequence having an RNA higher-order structure having a function for promoting a translation activity which comprises a base sequence selected from the group consisting of;

1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;

2) a base sequence containing the base sequence of 1);

3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;

4) a complementary strand of the base sequences of 1) to 3);

5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and

6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity.

4. (previously presented) A transformant that has been transformed with a recombinant vector containing a polynucleotide that is made up of one or more base sequences having a higher order structure having a function for promoting a translation activity, the sequence including a base sequence selected from:

1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

- 2) a base sequence containing the base sequence of 1);
- 3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;
- 4) a complementary strand of the base sequences of 1) to 3);
- 5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and
- 6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity.

5. (currently amended) A method for synthesizing a heterologous protein or a heterologous polypeptide utilizing a polynucleotide that is made up of one or more base sequences having an RNA higher-order structure having a function for promoting a translation activity, the base sequence including a base sequence selected from the group consisting of:

- 1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;
- 2) a base sequence containing the base sequence of 1);
- 3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;
- 4) a complementary strand of the base sequences of 1) to 3);
- 5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

6) ~~a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity wherein at least PK (pseudoknot) I, II, and III structures and a function for promoting a translation activity are maintained therein.~~

6. (currently amended) A method for synthesizing a heterologous protein or heterologous polypeptide utilizing a recombinant vector containing a polynucleotide that is made up of one or more base sequences having a higher-order structure having a function for promoting a translation activity, the sequence including a base sequence selected from according to claim 5, wherein the RNA higher-order structure is made up of a base sequence selected from the group consisting of:

- 1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;
- 2) a base sequence containing the base sequence of 1);
- 3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;
- 4) a complementary strand of the base sequences of 1) to 3);
- 5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and
- 6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity.

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

7. (currently amended) A method for synthesizing a heterologous protein or a heterologous polypeptide in a cell-free protein synthesis system wherein the synthesis is carried out using a recombinant vector containing a polynucleotide that is made up of one or more base sequences having a an RNA higher-order structure having a function for promoting a translation activity, the sequence including a base sequence selected from:

- 1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;
- 2) a base sequence containing the base sequence of 1);
- 3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;
- 4) a complementary strand of the base sequences of 1) to 3);
- 5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions, and
- 6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity wherein at least PK (pseudoknot I, II, and III structures and a function for promoting a translation activity are maintained therein, in a cell-free protein synthesis system.

8. (original) The method for synthesizing a heterologous protein or heterologous polypeptide according to claim 7, wherein the cell-free protein synthesis system uses a wheat

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

germ extract.

9. (previously presented) The method for synthesizing a heterologous protein or a heterologous polypeptide according to claim 5, wherein the synthesis is carried out without using AUG translation initiation codon.

10. (previously presented) A method for initiating synthesis of arbitrary heterologous protein or heterologous polypeptide from arbitrary codon which comprises step of changing a combination of base pairs that make up PK (pseudoknot) I, II, and III structures in a RNA high-order structure having a function for promoting a translation activity, the sequence including a base sequence selected from:

- 1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;
- 2) a base sequence containing the base sequence of 1);
- 3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;
- 4) a complementary strand of the base sequences of 1) to 3);
- 5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and
- 6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity.

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

11. (previously presented) The method for initiating the synthesis according to claim 10 wherein, one or more combination(s) of base pairs that make up PK I is changed, and a base pair maintained in the changed higher-order structure is utilized for said initiating synthesis of said arbitrary heterologous protein or said heterologous polypeptide from said arbitrary codon.

12. (previously presented) The transformant of claim 4 wherein, at least PK (pseudoknot) I, II, and III structures are maintained in the RNA higher-order structure.

13. (previously presented) The method for synthesizing a heterologous protein or a heterologous polypeptide of claim 6 wherein, at least PK (pseudoknot) I, II, and III structures are maintained in the RNA higher-order structure.

14. (previously presented) A method for synthesizing a heterologous protein or a heterologous polypeptide utilizing a transformant which has been transformed with a recombinant vector containing a polynucleotide that is made up of one or more base sequences having a higher-order structure having a function for promoting a translation activity, the sequence including a base sequence selected from:

- 1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;
- 2) a base sequence containing the base sequence of 1);
- 3) a base sequence that has at least about 50% of homology in sequence to the base

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

sequence of 1) and that has a function for promoting a translation activity;

- 4) a complementary strand of the base sequences of 1) to 3);
- 5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and
- 6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity.

15. (previously presented) The method for synthesizing a heterologous protein or a heterologous polypeptide according to claim 6, wherein the synthesis is carried out without using AUG translation initiation codon.

16. (previously presented) The method for synthesizing a heterologous protein or a heterologous polypeptide according to claim 7, wherein the synthesis is carried out without using AUG translation initiation codon.

17. (previously presented) The method for synthesizing a heterologous protein or a heterologous polypeptide according to claim 8, wherein the synthesis is carried out without using AUG translation initiation codon.

18. (previously presented) The recombinant vector according to claim 3, wherein at least PK (pseudoknot) I, II, and III structures are maintained in the RNA higher-order structure.

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

19. (previously presented) The transformant according to claim 4, wherein at least PK (pseudoknot) I, II, and III structures are maintained in the RNA higher-order structure.

20. (new) A method for synthesizing a heterologous protein or a heterologous polypeptide comprising the steps of

providing a protein expressing system,

providing a polynucleotide encoding the heterologous protein or heterologous polypeptide and a polynucleotide that promotes translation activity, wherein the polynucleotide that promotes translation activity has an RNA higher-order structure including PK (pseudoknot) I, II, and III structures, and

translating the polynucleotide encoding the heterologous protein or heterologous polypeptide in the protein expressing system to form the heterologous protein or heterologous polypeptide, wherein the translating is initiated and/or promoted by the polynucleotide that promotes translation activity.

21. (new) The method for synthesizing a heterologous protein or heterologous polypeptide according to claim 20, wherein the RNA higher-order structure comprises a base sequence selected from the group consisting of:

- 1) one of the sequences of SEQ ID NOs:1 - 7;
- 2) a base sequence containing a base sequence of 1);
- 3) a base sequence that has at least about 50% homology to the base sequence of 1)

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

and that has a function for promoting a translation activity;

- 4) a complementary strand of a base sequences of 1) to 3);
- 5) a base sequence hybridizing with a base sequences of 1) to 4) under stringent conditions; and
- 6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in a base sequences of 1) to 5) and that has a function for promoting a translation activity.

22. (new) The method for synthesizing a heterologous protein or heterologous polypeptide according to claim 20, wherein the RNA higher-order structure comprises a base sequence selected from one of the sequences of SEQ ID NOs:1 - 7.

23. (new) The method for synthesizing a heterologous protein or heterologous polypeptide according to claim 20, wherein the RNA higher-order structure comprises SEQ ID NO:1.

24. (new) The method for synthesizing a heterologous protein or heterologous polypeptide according to claim 20, wherein the RNA higher-order structure comprises a base sequence selected from one of the sequences of SEQ ID NOs:1 - 7, except that the base sequence contains an alteration in one or more combinations of base pairs that make up PKI so that polynucleotide that promotes translation activity is able to initiate translation activity of a heterologous protein or heterologous peptide without an AUG translation initiation codon.

U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement

25. (new) The method for synthesizing a heterologous protein or heterologous polypeptide according to claim 22, wherein the RNA higher-order structure comprises the base sequence of SEQ ID NO:1, except that positions 187 - 188 of the base sequence of SEQ ID NO:1 are cc instead of uu and positions 158 - 159 are gg instead of aa.

26. (new) The method for synthesizing a heterologous protein or heterologous polypeptide according to Claim 20, wherein the protein expressing system is a cell-free system.

27. (new) The method for synthesizing a heterologous protein or heterologous polypeptide according to Claim 26, wherein the protein expressing system is a wheat germ extract.

28. (new) The method for synthesizing a heterologous protein or heterologous polypeptide according to Claim 20, wherein the protein expressing system is a cell and wherein step of providing a polynucleotide encoding the heterologous protein or heterologous polypeptide and a polynucleotide that promotes translation activity is carried out by transforming the cell with a recombinant vector containing the polynucleotide encoding the heterologous protein or heterologous polypeptide and the polynucleotide that promotes translation activity.

29. (new) The method for synthesizing a heterologous protein or heterologous polypeptide according to Claim 28 wherein the polynucleotide that promotes translation activity

**U.S. Patent Application No. 10/088,750
Supplemental Response To Restriction Requirement**

is immediately upstream on the recombinant vector from the polynucleotide encoding the heterologous protein or polypeptide.